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**Memoirs of the
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in India**

**HELMINTHOSPORIUM SPP. ON CEREALS AND
SUGARCANE IN INDIA**

PART I

**(DISEASES OF ZEA MAYS AND SORGHUM VULGARE
CAUSED BY SPECIES OF HELMINTHOSPORIUM)**

BY

M. MITRA, M. Sc

First Assistant to the Imperial Mycologist



AGRICULTURAL RESEARCH INSTITUTE, PUSA

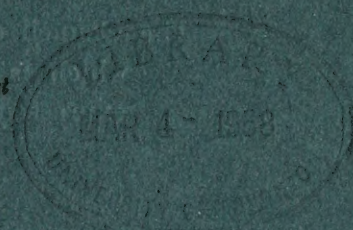
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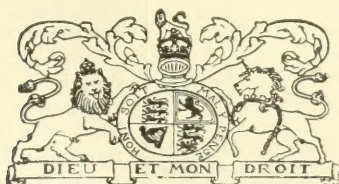
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HELMINTHOSPORIUM SPP. ON CEREALS AND SUGARCANE IN INDIA, PART I.

(DISEASES OF *ZEA MAYS* AND *SORGHUM VULGARE*
CAUSED BY SPECIES OF *HELMINTHOSPORIUM*.)

BY

M. MITRA, M.Sc.,
First Assistant to the Imperial Mycologist.

[Received for publication on 2nd June, 1922.]

1. Introduction.

MANY species of the genus *Helminthosporium* are common parasites on various members of the *Gramineæ*, and some species cause considerable damage to crops, e.g., "stripe disease of barley" and "blight of maize" are well known in America and Europe. Saccardo in his "Sylloge Fungorum" has recorded about 30 species on various *Gramineæ* and there are a few other species which have been recorded since the publication of the last volume. In India, most of the important crops such as wheat, oats, barley, maize, great millet (*jowar*), rice, *Eleusine*, *Panicum frumentaceum* and sugarcane are commonly attacked by species of *Helminthosporium*, and damage in some cases is considerable. They generally attack the leaves and ears and form light yellowish brown spots which in most cases coalesce and destroy the leaves.

The object of this study was to determine the range of the host plants of species parasitic on cereals and sugarcane and to ascertain whether (a) morphologically similar forms from different hosts vary in range of host, and (b) whether morphologically unlike forms have the same host range. An attempt was also made to secure the perfect stage by cultivating the species on various kinds of media.

The following species on cultivated cereals and sugarcane have been recorded from India¹: *H. Avenæ* Br. and Cav. (oats), *H. gramineum* Rabh. and *H. teres* Sacc. (barley), *H. turcicum* Pass. (maize and great millet), *H.*

¹ Butler, E. J. "Fungi and Disease in Plant," 1918.

nodulosum B. and C. (*Eleusine coracana* and *E. ægyptiaca*), *H. Sacchari* Butl. (sugarcane). Beside these, *H. Oryzæ* Miyabe and Hori (rice), *H. flagelloideum* Atk. (*Panicum*) and a species on wheat have been collected from various places. The species on wheat has been brought into culture from diseased wheat plants obtained from Burma, Nagpur, Poona and Pusa, and all cultures from these four places show a good deal of variation in cultural characters and in the measurement of spores. They all give positive results when inoculated on wheat and behave alike when inoculated on other hosts. Wheat *Helminthosporium* varies in culture on the same media, and when compared with *H. teres* Sacc. found on barley, appears to be a variety of this species. *Helminthosporium* has been also observed on some wild grasses such as *Cynodon*, *Andropogon*, *Panicum* and *Eleusine indica*. A detailed account of all these will be published later on.

The present paper deals with the species on *Zea Mays* and *Sorghum vulgare* (jowar) and gives an account of the growth in culture on different media and results of cross-inoculation experiments on other important cereals and sugarcane. A brief summary of this has been published in the "Scientific Reports of the Agricultural Research Institute, Pusa" ^{1,2}.

2. *Helminthosporium* on *Zea Mays*. (*H. turcicum* Pass.)

Helminthosporium turcicum Pass. is an important fungus parasite of maize in Bihar and also occurs in other parts of India wherever this crop exists, except in some dry places such as the Punjab; but the damage done is not great. It attacks the leaf to a great extent and also, in a less degree, the male inflorescence. In severe attacks both the size and out-turn of grain are smaller. In the early crop, *i.e.*, before the rains, the disease is very rare. In the late crop, the disease is fairly prevalent and nearly every plant is attacked to some extent. The lower leaves of old plants and those of young plants are easily attacked and the disease is found abundantly on them, particularly during heavy rains. In some parts of the world a fungus known by the name of *H. inconspicuum* C. and E. occurs on maize, but as the description agrees nearly with present species, it is probably identical with *H. turcicum* Pass. Other authors (Massee,³ Smith,⁴ Comes⁵ and Pammel⁶) consider that these two species are the same fungus.

¹ *Scientific Reports of the Agricultural Research Institute, Pusa*, 1919-20, pp. 62-64.

² *Annual Report, Board of Scientific Advice for India*, 1919-20, p. 31.

³ Massee, G. "Diseases of Cultivated Plants and Trees," 1910, p. 48.

⁴ Chester and Smith. "Notes on Fungus Diseases in Delaware." *Delaware Expt. Stat. Bull.*, 63.

⁵ Comes. "Crittogamia Agraria," 1891, p. 409.

⁶ Pammel, King and Bakke. "Two Barley Blights." *Bull. Iowa Coll. Stat.* 116, 1910.

HISTORY AND DISTRIBUTION.

The disease was first described in 1876 in Parma by Professor Passerini as occurring on leaves of maize. Since then it has been found in the north of Italy¹ and has been recorded in South Europe, France, Russia, England, the United States of America, Japan, China,² the Philippine Islands, New South Wales, Queensland, and India (Bihar,³ Dharwar, Almora and other parts of the United Provinces, Bengal and Burma).

In the United States of America it was noticed as early as 1889. In Southern France it was observed in 1903, in India (Bihar) in 1907, in South Africa in 1912, in Australia in 1915 and in the Philippine Islands in 1919. Most writers have merely recorded the disease and have made no attempt to prove the parasitism of the fungus.

Serious damages have been recorded from various countries, viz., South Africa³, the Philippine Islands^{4, 5, 6} and Italy⁷. Robinson⁸ recorded it from the Philippine Islands and Otto Reinking^{1, 5} described it (1919) as a common and at times very destructive disease in Philippine Islands. He says that the disease is more severe on newly introduced corns which are not acclimatized and are in a weakened condition and that native corn is not severely attacked. He found a black mould on the male inflorescence also which Saccardo has determined as *H. curvatum* but which resembles *H. turcicum* very closely.

It has been described as a serious and destructive disease in Delaware by Chester and Smith⁹ (1903) and by Ducomet⁹ (1903) in Southern France. Anon¹⁰ (1915) reports it to have caused severe loss in New South Wales in localities with heavy rainfall and hot steamy weather.

It is a difficult disease to check and no proper treatment is yet known. Smith believes that spores may be able to live after passing through the alimentary canal and so manure from an animal excreta may prove a source of infection. Anon recommends rotation and use of more leguminous plants.

¹ Loverdo, J. "Maladies Des Cereales," p. 279, 1892.

² Otto Reinking. "Diseases of the Economic Plants in Southern China." *The Philippine Agriculturist*, 1919, vol. VIII, no. 4.

³ Pole Evans. Report of Plant Pathologist and Mycologist in *Ann. Rep. S. Afr. Dept. of Agri.*, 1912-13.

⁴ Otto Reinking. "Philippine Economic Plant Diseases." *The Philippine Journal of Science, A.*, vol. XIII, no. 5, 1918, p. 251.

⁵ Otto Reinking. "Philippine Plant Diseases," *Phytopathology*, vol. IX, no. 3, 1919, p. 140.

⁶ Robinson. "Corn Leaf Blight in the Philippines." *Phil. Agri. Rev.*, IV, p. 350.

⁷ Loverdo, J. *loc. cit.*

⁸ Chester and Smith. "Notes on Fungus Diseases in Delaware." *Delaware Expt. Stat. Bull.* on. 63.

⁹ Ducomet, V. "The Browning of Maize in France." *Jour. Agri. Prat., N. Ser.*, 5 (1903), no. 16, pp. 507-511. (Only a abstract seen in *Expt. St. Rec.* of U. S. A.)

¹⁰ Anon. "Blight in Maize." *Agri. Gaz. N. S. Wales*, 26 (1915), no. 5, p. 338.

THE SYMPTOMS OF THE DISEASE.

The common terms applied to this disease are "blight of maize," "blight of corn" and "leaf spots." Ducomet¹ has suggested the name of "brulure" for the disease on account of the peculiar browning burnt condition of the leaves. Maize blight, however, seems to be the most fitting term for the disease.

The disease makes its appearance, when the plant is quite young, in the form of small yellowish spots which rapidly increase in size and take on a pale brown colour. In the beginning, the infected area on the leaf is minute, roundish, and pale brown, and gradually increases in size becoming somewhat oval, the long axis of the spots being parallel to the vein. They may coalesce covering a large surface of the leaf. These spots, the breadth of which is often limited by leaf veins, later on, become somewhat translucent and to the naked eye appear like a greyish green mould on account of the conidia and conidiophores. Conidiophores and conidia are found all over the spot on both sides of the leaf but in a greater number in the central atrophied portion of the diseased area. The spots, later on, become dry and brittle and the young leaves are easily killed (Plate I, fig. 1).

In the male inflorescence the disease assumes a blackish mould-like appearance on the surface of the glumes of male spikelets. The attack is not extensive and scattered spikelets here and there are infected (Plate I, fig. 2). The mycelium ramifies within the tissues of the paleæ and the stamens.

ETIOLOGY OF THE DISEASE.

The constant association of the fungus with the disease leaves little doubt that *H. turcicum* is the cause of maize blight. Healthy plants, when inoculated, develop the disease and produce typical spores which resemble those from the field.

Leaves of maize were inoculated several times with pure culture obtained from diseased maize leaves, and it was found that the fungus had penetrated the tissues and formed spots as in nature. In some cases the infection took place within 24 hours. The plants inoculated and the controls were kept covered either with bell-jars or in moist glass chambers. It was observed that infection was rapid and vigorous on young plants and on lower leaves of mature plants. Inoculations were also made on the male inflorescence and it was noticed that all spikelets inoculated had been infected and infection had spread also on the neighbouring spikelets.

¹Ducomet, V. "The Browning of Maize in France." *Jour. Agri. Prat. N. Ser.*, 5 (1903), no. 16, pp. 507-11. Only *abstrach* seem in (*Expt. St. Rec. of U. S. A.*)

A strain from the male inflorescence of maize was also isolated and inoculations were done on the leaves and spikelets of male inflorescences. All were infected and produced the disease as in fields. In this case all inflorescences inoculated were either enclosed in glass chimney or the plants were kept in moist chambers. Controls were kept on all occasions and remained healthy.

Seedlings of maize were inoculated with a pure culture from leaves. The inoculation was done on the roots, grains and on the sheath and near the tip of the growing seedlings; within two days, in most cases, the sheath became yellowish brown and mycelium was found inside it. Later on, on examination, it was found that the mycelium had penetrated roots, pericarp and testa, and in some cases the aleurone layers of grains and also hypocotyl, etc. Most of the seedlings died on account of the disease but those which remained alive showed no growth of fungus in their tissue after some time. This shows that the fungus is capable of killing and destroying germinating grains and young seedlings but the infection is localized and does not keep pace with the growth of the host as in smut. Details of inoculations are given in the following table:—

TABLE I.
Summary of the inoculations on maize with H. turcicum Pass.

No.	Date	Strain used	Place inoculated	No. of inoculations	No. of successful inoculations	No. of control	Control infected	REMARKS
1	19-7-18	Maize leaf	Both sides of leaves	12	12	2	..	Five leaves were wounded and seven unwounded. Leaves unwounded.
2	30-7-18	Do.	Do.	15	15	1	..	
	5-8-18	Do.	Do.	9	9	1	..	
	8-8-18	Do.	Do.	4	4	1	..	
3	28-8-19	Do.	Do.	10	10	1	..	Do.
4	5-10-19	Maize male inflorescence	Do.	9	9	1	..	Do.
5	17-10-19	Do.	Male inflorescence	13	13	1	..	Do.
	19-10-19	Do.		15	15	1	..	Do.
6	19-10-19	Maize leaves	Do.	15	15	1	..	Do.
7	28-10-19	Male inflorescence	Do.	40	30	1	..	Do.
8	10-6-20	Leaf	Leaves	6	5	1	..	Do.
	7-8-20	Do.	Do.	11	5	1	..	
	10-12-20	Do.	Do.	11	10	1	..	
9	4-8-19	Do.	Seedlings	22	22	10	..	
Total				192	174	24	..	91%

The above experiments furnish proof of the pathogenic nature of *H. turcicum* on maize leaves and inflorescence and show that the fungus on leaves and on the male inflorescence of tassel is identical.

MORPHOLOGY OF THE PARASITE.

Mycelium. The mycelium of the parasite ramifies in the tissues of the leaf and thus destroys the assimilatory apparatus of the plant, weakening the plant so that its growth is checked. The spots in which the mycelium spreads later on become dry and brittle. The mycelium consists of branched septate hyphæ, the cells of which sometimes become irregularly swollen. It is both intra- and inter-cellular. Inside the matrix the mycelium is subhyaline but where conidiophores arise it is light to olive brown colour.

In culture a light greyish green colour appears which increases gradually, becoming darker. In old cultures the cells swell up, the cell wall becoming somewhat thick and thus give rise to thick-walled cells resembling chlamydospores. Spore formation takes place in cultures 6 or 7 days old. Conidiophores bear conidia at the apex. Sometimes conidia are formed with such rapidity that at the tip of conidiophore 3 or 4 are seen. These at first sight appear to be borne in cluster, but when observed carefully it is found that only the youngest is at the tip while the rest are just below in acropetal succession. Later on, as the conidiophores elongate, the distance between the spores increases and they become more and more lateral. The conidiophore becomes bent in several places where conidia are attached, but when spores are formed vigorously one after the other no such bending is noticed. Sometimes in culture no spore formation takes place, this is especially the case when the fungus has been cultivated for a very long time in one kind of medium, but when the medium is changed, spores are formed again. On water culture or on the culture kept in a drop of water in a moist chamber abundant spore formation takes place. This method was employed to induce the fungus to form spores when it was sterile in culture tubes.

The fungus loses its parasitic nature if cultivated for a long time in culture ; for instance, a fresh culture which was able to infect leaves vigorously was kept in culture for nearly a year, it was then used for inoculation but no infection took place. Fresh cultures which were exactly like the cultures of last year were again taken from the field and when inoculated gave successful results. Conidiophores are simple, septate and erect. They arise in clusters on either side of the leaf, are sometimes slightly bent above and are light brown in colour. They are 115 to 150 by 6 to 8.7 μ in diameter and come out either through stomata or by directly piercing the epidermis (Plate II, figs. 18-21).

Conidia are fusiform or spindle-shaped, pointed, a little curved, light greenish brown, 4-7 septate and 75 to 125 by 18 to 24 μ in diameter (Plate II, figs. 6-17). The breadth varies in different localities; for instance, spores from Almora specimens were up to 26.6 μ in breadth while those from Pusa were up to 24 μ only. (Almora 72 to 117.8 by 19 to 26.6 μ , Malda (Bengal) 35 to 130 by 17 to 24 μ . Spores from Burma and Dharwar specimens are like those from Almora.)

There is some variation with regard to the shape of spores. Typical spores of *H. turcicum* Pass. are spindle-shaped and pointed^{1,2}, and these have been observed in specimens from various localities in India, such as Almora, Dharwar and Burma (Plate II, figs. 14-17), but in Pusa and Malda (Bengal) specimens, the spores are somewhat curved and narrow (Plate II, figs. 7-13). The spots on the surface of leaves are however alike in specimens collected from various localities. Though the spores in Pusa and Malda specimens are somewhat more curved yet in cultures the conidia are generally straight, spindle-shaped, and very few are curved. Conidia of the fungus from the glumes of male spikelets are a little longer and more curved than those of the leaf fungus and the fungus has been named *H. curvulum* Sacc. In the Philippines³ it is described by this name but here in cultures it resembles the strain from the leaf and, when inoculated on the leaves, produces the same kind of spots as the leaf strain.

CULTURES.

Spore germination. When a few fresh spores are placed in a hanging drop culture, germination takes place within few hours. Germ tubes generally protrude from the ends of the spore but they have also been noticed to come from any of the middle cells of the spore (Plate II, figs. 1 and 3). In some cases the internal partition of the spore breaks down and a continuous tube is formed. Again, in a few cases, the septa disappear before the protrusion of the germ tube, while in other cases it was noticed that the septa near the ends only were disorganized (Plate II, fig. 4). Plate II, figs. 1 and 2-6 were drawn after 48 and 18 hours of sowing respectively. Detached conidiophores are also capable of germinating by giving out a tube from either end and have often been noticed to infect the leaves.

Pure cultures were obtained several times by the poured plate method or from a diseased portion of a leaf after washing it for a few minutes in a solution

¹ Saccardo. *Fungi ital.*, a fig. 824.

² Massee. "Diseases of Cultivated Plants and Trees," 1910, p. 481.

³ Otto Reinking. "Philippine Plant Diseases." *Phytopathology*, vol. IX, no. 3, 1919, p. 140.

of corrosive sublimate in water (1 in 1000 c.c.) and then in distilled water. The washed piece of diseased leaf was transferred to an agar tube. This gave rise to an aerial growth in the tube from which a subculture was taken giving rise to pure culture.

In the beginning spores are produced laterally on the mycelium in a large number. When young they appear to be sessile but after four or five hours' growth they are distinctly seen on a stalk which grows in length and bears many more conidia. Very often the conidia are borne at the tip of the hyphæ but after some time each of them becomes stalked, the stalk increases in size and forms two conidiophores.

On some media gemmæ-like bodies are formed and on some irregular stromatic masses, but no sclerotia or pycnidia have ever been observed.

Growth on different culture media. A large number of media were inoculated in duplicate with pure culture of *Helminthosporium*. All the media were inoculated on the same day and from the same tube. On some of the media the fungus was grown repeatedly to mark variations, if any. Tubes were kept for more than six months and in some cases for almost a year but no perfect stage of the fungus was obtained.

The following is a detailed account of the growth on various media:—

(1) *Nutrient plain agar* (+4 Fuller's scale).
 (2) *Dextrose agar* (+2 " ").
 (3) *Glycerine agar* (+6 " ").
 (4) *French bean agar* (+8 " "). The growth is poor in all these media with very little aerial growth. Spore formation is scanty and spores are smaller than those formed on other media.

(5) *Litmus lactose agar* (+6 Fuller's scale). The growth is submerged and creeping in the beginning but later on a woolly aerial growth appears. After a few days the pinkish medium gradually turns blue. There is a good deal of spore formation, but the size of spores is smaller as on last medium. Spores are as small as 15μ in length and septation is reduced very much in most cases and in some cases spores are formed without any septum. Average spores are 45.6 to 95 by 15.2 to 22.8μ in diameter. Mycelium becomes gemmate in old cultures.

(6 & 7) *Nutrient glucose agar* (+5 Fuller's scale) and *nutrient saccharose agar*. Fairly good growth of a greyish green colour. Spore formation is copious. The medium gradually becomes greenish, then dark green on account of the submerged growth, and after some days still darker. Spore formation is more copious at $20-22^{\circ}\text{C}$. than at $30-32^{\circ}\text{C}$., and at the later temperature sometimes spores of abnormal size are formed. At low temperatures growth is slow and the colour is dark greenish grey instead of light greenish grey as at 30°C . It takes about four days to fill the tube completely at 30°C ., but spore formation decreases gradually with the increase of temperature, i.e., when growth is increased, spore formation is less and *vice versa*. In old cultures the mycelium swells up irregularly forming gemmate.

(8) *Thaxter's hard potato agar* (+6 Fuller's scale). Copious aerial growth, the medium becomes greyish green. The growth is light green at 32°C ., and dark at 22°C . In 5 or 6 days the tube is quite full and the mycelium is seen spreading on the walls of the tube. Spore formation is very free and spores are greenish brown in colour. Hyphæ light greenish brown

and the submerged mycelium is green to dark green. Spore formation is more rapid at a low temperature.

(9) *Potato juice agar* (+6 Fuller's scale). Growth not well developed. Hyphæ cells show irregular swelling here and there. Spore formation is poor. Submerged growth greenish and aerial greyish in appearance.

(10) *Coom's synthetic agar* (+8 Fuller's scale). The growth is quite good with woolly aerial mycelium of light greyish green colour. The medium also becomes greenish, spore formation is copious. Mycelium later on turns gemmate.

(11) *Cellulose agar* (+9 Fuller's scale). The growth is poor and almost submerged, of greyish colour. Spore formation fair. The colour of medium does not change.

(12) *Starch agar* (+4 Fuller's scale). Fair woolly growth, aerial hyphæ arise in clusters and grow radially. The medium turns light green. Spore formation is good.

(13) *Corn-meal agar* (+2 Fuller's scale). The growth is best on this medium. Copious, woolly, greyish green aerial growth at 30°C. The medium turns greenish and then dark green on account of submerged growth. In four days at 30°C. the growth is very profuse and the tube is almost full, the mycelium spreading on the walls of the tube. Spore formation is very good especially at a low temperature at which growth is slow. Hyphæ light olive green.

(14) *Oat-meal agar* (+2 Fuller's scale). Copious woolly aerial growth of greenish grey colour. The medium becomes dark green near the margin. The submerged growth is also greenish and then turns darker gradually. Spore formation fairly large in number and growth is more rapid at 30°C. than at 20-22°C.

(15) *Wheat-meal agar* (+2 Fuller's scale). Copious aerial growth, the mycelium spreading on the walls of the tube and lower portion of the tube quite full. Hyphæ olive green. Abundant spore formation.

(16) *Rice-meal agar* (+4 Fuller's scale). Growth good, but little less than that on corn-meal agar. Copious spore formation. Lower portion of the tube quite full with mycelium. Medium turns light green.

(17) *Barley-meal agar* (+3 Fuller's scale). Very good woolly aerial growth. Mycelium looks dark greenish and hyphæ light olive green. Abundant spore formation as on rice-meal agar. It is one of the best media for the growth and production of spores.

(18) *Jowar-meal agar* (+2 Fuller's scale). A good growth with abundant aerial mycelium. The medium gradually turns green and then dark green. Spore formation good especially at 20-22°C. Growth slow at low temperature. Spores generally are 60.8 to 140.6 by 19 to 26.6 μ in diameter with 3 to 7 septa. Sometimes spores are very small and with few or no septum. Those abnormal spores are not more than 60 μ in length and 10.4 μ in breadth and are formed when kept at a little high temperature.

(19 & 20) *Sugarcane leaf juice agar* (+4 Fuller's scale) and *rice leaf juice agar* (+3 Fuller's scale). In both of these media growth is poor and submerged. Spore formation is scanty.

(21) *Maize leaf juice agar* (+3 Fuller's scale). Fair growth with abundance of spore formation.

(22, 23 & 24) *Sterilized maize stem, sterilized sugarcane megasse and sterilized paddy straw*. There was a good production of spores on all, especially on sterilized maize stem and sterilized paddy straw. The cultures were kept for about 7 months in each case but there was no sign of the appearance of the perfect stage.

(25) *Sterilized wheat straw*. A good deal of spore formation took place and greyish green mycelium was formed in the water at the bottom of the tube. No further growth of the fungus, however, took place though the tubes were kept for more than a year.

(Note. Tubes in 22-25 were kept at two temperatures, viz., 22°C., and at room temperature of about 27-30°C. The growth was the same at both temperatures.)

The growth was best on corn-meal agar, barley-meal agar, rice-meal agar, Thaxter's hard potato agar and jowar-meal agar respectively.

EFFECT OF REACTION OF CULTURE MEDIA.

This experiment was run in duplicate on glucose medium. Twenty tubes were inoculated, two of each reaction and ranging from—15 to +30 Fuller's scale. After three days there was found to be no growth at +25 and +30 Fuller's scale; very poor at +20, and poor at +15 and—15. It was little at -10 and +10, good at +5 and best at 0 Fuller's scale. In eight day-old culture the growth increased at +10 and +5 but comparatively little took place at 0. From the above data it is observed that this fungus prefers reaction from a neutral to an acid media ranging from 0 to +10 Fuller's scale and is best at +5 Fuller's scale though growth is more rapid at 0 in the beginning.

LONGEVITY OF THE SPORES OF MAIZE HELMINTHOSPORIUM.

Large numbers of diseased spots of maize *Helminthosporium* were cut and put in a stoppered bottle and kept for more than a year. It was noticed that up to four months ninety per cent. of the spores germinated when placed in a drop of water and incubated in a moist chamber, and up to eight months fifty per cent; after eight months the spores rapidly lost their power of germination, and after a year they were incapable of germinating. This shows that spores can survive long enough to infect the next crop. It is quite probable that this fungus occurs on some wild grasses also and a search for them is being made.

RELATION OF PARASITE TO HOST. INFECTION AND CONIDIOPHORE PRODUCTION.

The penetration of the fungus takes place either through stomata or by directly piercing an epidermal cell (Plate III, figs. 1, 2 and 3). It can penetrate any epidermal cell, but generally it enters through subsidiary cells. The hypha from a germinating spore comes in contact with the cuticle, swells up and then sends out a narrow tube. This pierces the cuticle and may travel for a distance inside the cuticle, parallel to the outer wall of the epidermis, and after some time either enters an epidermal cell or passes through the side walls of two epidermal cells into the cells below. Sometimes it surrounds an epidermal cell, sending in branches which fill the cavity. When penetration is through a cell in contact with a guard cell, it enters the sub-stomatal space, branching freely, and becomes septate. It passes to other cells directly or through two adjacent cell-walls, *i.e.*, it is both intra- and inter-cellular. The mycelium branches and sometimes forms a net work below the epidermal layer. A few hyphae may enter an epidermal cell to form a stromatic mass from which conidiophores arise. The development of fructifications depends upon

the amount of moisture in the air and may take place after 5 or 6 days of infection.

When the fungus attacks a spikelet, the glumes, paleæ and anthers are all infected. It forms a superficial mycelium on the surface of the glumes on which a good deal of spore formation takes place.

CROSS-INOCULATION EXPERIMENTS.

Maize *Helminthosporium* was cross-inoculated on *Sorghum*, rice, wheat, oats, barley, *bajra* and sugarcane. The inoculation was done on leaves, and plants were either covered with bell-jars or placed in big glass cages where there was sufficient moisture. After infection took place the bell-jars or glass cages were removed. In every case a control was kept. The following table shows the result of experiments :—

TABLE II.

Cross-inoculations on Sorghum.

No.	Date	No. of inoculations	No. infected	Control	Control infected	REMARKS
1	30-7-18	8	4	1	..	The leaves were wounded.
2	5-8-18	8	4	1	..	
3	5-8-18	2	2	1	..	
4	11-8-18	8	3	1	..	
5	18-8-18	10	6	1	..	
6	13-8-19	3	2	1	..	The plants were kept in a warm and moist room.
7	10-6-20	11	8	..	..	
8	7-8-20	11	6	..	..	
9	11-12-20	18	14	..	..	
TOTAL		79	49	..	..	62%

Out of 79 inoculations made, 49 were successful. Controls kept showed no sign of disease. The spots formed were like those of *jowar Helminthosporium* on *jowar*. There was always a good deal of spore formation on both sides of leaves. Maize *Helminthosporium* however spreads less rapidly on *jowar* than *jowar Helminthosporium* on *jowar*.

TABLE III.
Cross-inoculations on sugarcane.

No.	Date	No. of inoculations	No. infected	Control	Control infected	REMARKS
1	5-8-18	7	4	1	..	In most cases the inoculated leaves were enclosed in a chimney, both ends of which were plugged with cotton wool so that the inoculated portions were as if in a moist chamber.
2	11-8-18	7	6	1	..	
3	18-8-18	18	10	1	..	
4	13-8-19	20	15	1	..	
5	29-8-19	10	5	1	..	
Total		62	40	..	..	64%

Out of 62 inoculations made, 40 were successful. Maize *Helminthosporium* when inoculated on sugarcane formed small dirty straw coloured spots which increased in size when leaves were kept moist. Though maize *Helminthosporium* is capable of infecting sugarcane leaves (Plate III, figs. 4 and 5), yet they are not identical.

TABLE IV.
Cross-inoculations on rice.

No.	Date	No. of inoculations	No. infected	Control	Control infected	REMARKS
1	11-11-19	24	3	1	..	
2	25-11-19	8	2	1	..	
3	29-11-19	8	4	1	..	
4	10-6-20	10	3	1	..	
5	7-8-20	11	3	1	..	
Total		61	15	..	..	24%

Maize *Helminthosporium* when inoculated on rice infects very few of the leaves and in these also the fungus after entering spreads very little and a spot is formed very rarely. Of 61 inoculations made, infection took place only in 15, and out of these 15 very few formed spots with conidiophores and spores.

The above table shows a low proportion of successful infection and in these cases penetration was slight. Rice may almost be considered to resist the attack of maize *Helminthosporium* though sometimes spots are formed under laboratory conditions.

TABLE V.
Cross-inoculations on bajra.

No.	Date	No. of inoculations	No. infected	Control	Control infected	REMARKS
1	29-11-19	20	..	1	..	0%

No infection on this plant took place.

TABLE VI.
Cross-inoculations on wheat.

No.	Date	No. of inoculations on leaves	No. of leaves infected	No. of inoculations on ears	No. of ears infected	Control	Control infected	REMARKS
1	19-1-20	12	12	..	..	1	..	
2	31-1-20	7	4	..	..	1	..	
3	6-2-20	10	10	..	..	1	..	
4	3-2-21	12	9	5	5	1	..	The leaves and ears of same plants were inoculated.
Total		41	35	5	5	..	..	87%

Maize *Helminthosporium* can infect wheat easily. Out of 46 inoculations made, 40 were successful. The infected leaves became straw-coloured and the spots gradually increased in size and ultimately killed the leaves. A good deal of spore formation took place on leaves and on the ears inoculated.

TABLE VII.

Cross-inoculations on barley.

No.	Date	No. of inoculations	No. infected	Control	Control infected	REMARKS
1	19-1-20	10	5	1	..	
2	25-1-20	11	9	1	..	
3	11-4-20	8	2	1	..	
4	3-2-21	16	6	1	..	
	Total	45	22	..	..	48%

Maize *Helminthosporium* can infect barley but the infection is not so vigorous as that on wheat. Out of 45 inoculations, 21 were infected. Spores were formed on both sides of the leaves inoculated.

TABLE VIII.

Cross-inoculations on oats.

No.	Date	No. of inoculations	No. infected	Control	Control infected	REMARKS
1	19-1-20	8	0	1	..	
2	25-1-20	12	9	1	..	
3	6-2-20	12	9	1	..	
4	11-4-20	8	6	1	..	
5	3-2-20	13	3	1	..	
	Total	53	27	..	..	51%

Out of 53 inoculations made, 27 were successful. The infected spots gradually increased in size and spore formation took place. Sometimes no infection took place as on those inoculated on 19th January, 1920.

The above cross-inoculation experiments show that maize *Helminthosporium* can infect most of the cereals and sugarcane when inoculated artificially

in the laboratory, *bajra* is altogether immune and rice is affected very little. The following is a summary of the results of inoculations :—

Maize 91%	Sugarcane 64%	<i>Jowar</i> 62%
Wheat 87%	Oats 51%	Barley 48%
<i>Bajra</i> 0%	Rice 24%	

3. *Helminthosporium* on *Jowar* (*Sorghum vulgare*).

Jowar Helminthosporium has been collected from very few places in India and observed only in the Punjab (Amritsar and Lyallpur). The damage done by this fungus to the crop is very slight. Morphologically the fungus is identical with that on maize but it must be a different strain or variety as indicated by the cultural characters. Besides it has been observed in Bihar that this fungus is common on maize but absent on *jowar*, while in the Punjab *Helminthosporium* has been found on *jowar* and not on maize. In Bihar very often maize and *jowar* are grown as a mixed crop, and the leaves of maize are found to be diseased but not those of *jowar*. But when maize *Helminthosporium* is cross-inoculated on *jowar* in the laboratory, infection takes place. All these points, together with the difference in cultural characters, indicate that *Helminthosporium* on *jowar* is a different strain or variety of *H. turcicum* Pass.

This fungus has been reported from other countries¹, viz., Egypt, China, etc., where it is not very common or injurious to this host.

There are also other species of *Helminthosporium* known on this host, such as *H. Cookei* Sacc.^{2, 3}, *H. Sorghi* Schw.^{2, 3} and *H. geniculatum* E. and T.⁴, and lastly *H. inconspicuum* C. and E. which is probably the same as *H. turcicum* Pass.

THE SYMPTOMS OF THE DISEASE.

The fungus is found on leaves only and forms long, narrow spots of dirty brown or straw colour (Plate I, fig. 3). The spots are marked from the healthy tissue by a reddish ring. Generally infection takes place from the tip and extends downward either along the margin or along the midrib. Very often two or more spots are formed along the edge and these coalesce and form into one. Sometimes long elliptical or elongated spots are formed on the blade of the leaf. If these are in large number, they unite and form one irregular spot.

¹ Butler, E. J. "Fungi and Disease in Plants," 1918.

² Saccardo. *Sylloge Fungorum*, vol. IV, pp. 420 and 423.

³ Rabenhorst. *Kryptogamen Flora*, vol. IX, pp. 37 and 909.

⁴ Palm Bj. "De *Helminthosporium* Ziekten," p. 16. *Dept. Landb. Nijv. en Handel., Meded. Lab. Plantenziekten*, no. 34 (1918). Eenige Ziekten, Waargenomen aan de tarme op Java.

ETIOLOGY OF THE DISEASE.

The parasitic nature of the fungus was proved by the following inoculation experiments.

TABLE IX.

Inoculations on the leaves of jowar with Helminthosporium isolated from the diseased leaves of jowar.

No.	Date	No. of inoculations	No. infected	Control	Control infected	REMARKS
1	19-11-19	24	19	9	..	
2	24-11-19	9	9	1	..	
3	30-1-20	6	6	1	..	
4	21-5-20	19	11	1	.	
5	10-6-20	18	14	1	..	Reisolated from No. 4 and inoculated.
6	7-8-20	10	10	1	..	
7	11-12-20	11	8	1	..	Plants kept in a moist warm room.
Total		97	77	..	..	79%

When *jowar Helminthosporium* is inoculated on *jowar* the spots formed are typical and resemble those found in nature. The fungus was reisolated from an inoculated spot, taken into culture and again inoculated and the same symptoms and results were obtained. This proves the parasitic nature of the fungus.

MORPHOLOGY OF THE PARASITE.

The mycelium of this fungus is found in the matrix of the infected portion of the leaf. It is present also in the vessels of xylem. Very often they form stromatic masses in the epidermal cells.

Conidia are formed on both sides of the spot and conidiophores arise singly or in cluster of three or more emerging from stomata (Plate III, figs. 12-16). Conidia are 57 to 136 by 19 to 26 μ in diameter (Plate III, figs. 7-11). *Jowar Helminthosporium* resembles more the *Helminthosporium* on maize from Burma, Almora and Dharwar than that from Pusa and Malda. The spores of *jowar Helminthosporium* are straight, spindle-shaped and resemble those on maize from Burma, Almora and Dharwar, but spores from Pusa specimens are

a little curved and narrower and so differ somewhat from *jowar Helminthosporium* and maize *Helminthosporium* from Burma, Almora and Dharwar. The external characters, *i.e.*, spot formation and colour, etc., in Burma, Almora, Dharwar, Pusa and Malda specimens are the same, but in culture Pusa maize *Helminthosporium* has spores which are not so curved.

Conidiophores on *jowar* are more straight and bent at the tip but in the case of maize some are straight while others bent in a zig-zag manner. Maize conidiophores are slender and longer while those on *jowar* are thicker.

CULTURES.

A careful search was made in Pusa and in the neighbouring places in 1918-20 but no *Helminthosporium* was found on this host. Later on it was obtained from the leaves of *jowar* from the Punjab (Amritsar and Lyallpur) where this fungus is generally found after the rains. The culture was taken as in the case of maize fungus.

Spore germination is like that of maize *Helminthosporium* and needs no description.

Growth in different cultural media. The fungus was cultivated on a large number of culture media, and in many cases this fungus and maize *Helminthosporium* were grown together on the same kind of media for comparison. The fungus can grow well on most of the media tried and produces a large number of spores.

The fungus resembles maize *Helminthosporium* in most respects. The formation of conidia on conidiophores and the other details are somewhat like those of maize *Helminthosporium*. Though this fungus resembles the one on maize in the formation of spores, etc., and produces the same colour on the medium, there is a constant difference, *viz.*, that *jowar Helminthosporium* never produces a copious aerial growth on any of the media tried, and there is always a tendency to produce more of submerged and creeping mycelium, while in the case of maize there is a good aerial growth. On the other hand, this fungus always produces a very large number of conidia and sometimes the spore formation is so abundant that the contents of the mycelium are almost used up. The following media were tried:—

1. *Nutrient glucose agar.* Growth almost submerged and of dark greyish green colour with very little aerial growth. The growth is little more at 30°C. than at 20-22°C. with a good deal of spore formation. Conidia on this medium are 70 to 122.5 by 17.5 to 22.7 μ while those of maize *Helminthosporium* on the same medium are 63 to 126 by 15.8 to 22.7 μ in diameter.

2. *French bean agar.* The growth on this medium resembles very much that of maize *Helminthosporium* but *jowar Helminthosporium* is darker. The growth at 30°C. is more than that at 20-22°C. There is a good deal of spore formation.

3. *Wheat-meal agar.* The growth is like that of maize fungus but darker. Later on submerged and creeping dark greyish green mycelium is formed at 30-32°C. In the case of

maize *Helminthosporium* the colour is light greyish green but here it is dark greyish green and moreover aerial growth is absent. After some time little aerial growth appears but still the growth is darker than that of maize. Spore and mycelium resemble somewhat maize *Helminthosporium*. About 50 per cent. of *jowar* spores resembles the maize fungus but the remaining spore percentage is somewhat broader. The number of septa is the same in both.

4. *Jowar-meal agar*. There is a creeping and submerged growth of light greenish colour at 30°C., and crowded and compact at 22°C. The colour of medium becomes similar to maize fungus but afterwards it becomes dark green. After 10 or 12 days little aerial growth of dark greyish green colour appears. Spore formation is good.

5. *Litmus lactose agar*. The growth is poor and dark and greyish green, submerged and creeping in the beginning. The submerged portion becomes dark. Later on a little aerial growth appears. The growth on this medium is exactly like that of maize *Helminthosporium* on it. Spores are 45.6 to 95 by 15.2 to 22.8 μ in diameter. The spores and mycelium resemble maize *Helminthosporium* on this medium, the size, form and colour being alike.

6. *Coon's synthetic agar*. Fairly good at 30–32°C. of dark green colour. The growth resembles to some extent maize *Helminthosporium* on this medium. The fungus is more dark green at 22°C. Spore formation poor and the old mycelium becomes gemmate. The mycelium is darker than that of maize *Helminthosporium*.

7. *Corn-meal agar*. Growth dark greyish green at 22°C., and light at 32°C. Submerged growth is greenish and there is no aerial growth. Later on little aerial growth appears which is very much like that of maize and of greenish grey colour. The mycelium and spores resemble those of maize *Helminthosporium*. After 10 or 12 days maize and *jowar* cultures resemble very much on this medium. Spores and mycelium almost alike and spore formation more at a low temperature of 20 to 22°C. than at 30–32°C. Spores are 80.5 to 122.5 by 15.8 to 21 μ in diameter while those of maize *Helminthosporium* are 77 to 122 by 15.8 to 19 μ .

8. *Oat-meal agar*. Dark creeping growth at both temperatures but little more at 32°C. After some days growth appears dark greyish green, creeping and submerged. Spore formation is good. The mycelium resembles that of maize *Helminthosporium*. On this medium the aerial growth is comparatively smaller.

9. *Nutrient saccharose agar*. Creeping and submerged growth of dark grey colour with little aerial growth and poor spore formation.

10. *Thaxter's hard potato agar*. Dark green, submerged and creeping, resembling the growth of maize *Helminthosporium* but there is no aerial growth. After some time little aerial growth appears. Spore formation resembles that of *Helminthosporium* on maize.

11. *Wheat straw*. Wheat straw was sterilized in ordinary tubes containing little water. The fungus was grown on it and the tubes were kept at 27–30°C., and some at 22°C. There was a good production of spores on it. The growth was like that of maize, but in the latter case more spores were formed. In these, there was no good aerial growth. Greyish green mycelium was formed at the bottom of the tube in water.

From the results of inoculation experiments and from cultural characters this fungus appears to be very closely related to or identical with *Helminthosporium* on maize, but the fact that in one place it occurs on one host and not on the other, makes one to consider them as two different varieties of *H. turcicum* Pass.

CROSS-INOCULATION EXPERIMENTS.

The following cross-inoculations were made with *jowar Helminthosporium* on the same hosts on which maize *Helminthosporium* was inoculated and in the same manner.

TABLE X.

Cross-inoculations on maize.

No.	Date	No. of inoculations	No. infected	Control	Control infected	REMARKS
1	29-11-19	8	5	1	..	
2	30-1-20	10	6	1	..	
3	21-5-20	10	4	1	..	
4	10-6-20	14	10	1	..	
5	7-8-20	10	5	1	..	
6	11-12-20	10	..	1	..	
Total		62	30	..	..	48%

Forty-eight per cent. of inoculated maize were infected and produced spots which resembled very much the spots formed by the maize *Helminthosporium* on maize. It appears that maize *Helminthosporium* and jowar *Helminthosporium* are the same, but with slight differences in cultural characters and in the form of spores.

TABLE XI.

Cross-inoculations on sugarcane.

No.	Date	No. of inoculations	No. infected	Control	Control infected	REMARKS
1	29-11-19	10	8	1	..	No infection took place probably on account of low temperatures prevailing at the time.
2	11-12-20	10	0	1	..	
Total		20	8	..	..	40%

Forty per cent. of inoculated sugarcane leaves were infected. The spots formed were small, oval and, later on, dirty brown with brownish rings. They gradually increase in size, more in the longitudinal direction, and produce conidia and conidiophores which have normal character.

TABLE XII.
Cross-inoculations on bajra.

No.	Date	No. of inoculations	No. of successful inoculations	Control	Control infected	REMARKS
1	29-11-19	20	0	1	..	0%

No infection took place. It may be mentioned here that maize *Helminthosporium* also had no effect on *bajra*.

TABLE XIII.
Cross-inoculations on paddy.

No.	Date	No. of inoculations	No. of successful inoculations	Control	Control infected	REMARKS
1	29-11-19	10	2	1	..	
2	20-6-20	10	2	1	..	
3	7-8-20	10	0	1	..	
Total		30	4	..	..	13%

Out of 30 inoculations made, 4 were infected and these also showed simply penetration and very little spreading in the tissue of rice leaves. The growth ceases soon and there is no spore formation. Maize *Helminthosporium* also behaves like this on paddy.

TABLE XIV.
Cross-inoculations on wheat.

No.	Date	No. of inoculations	No. of successful inoculations	Control	Control infected	REMARKS
1	25-1-20	14	5	1	..	
2	6-2-20	10	10	1	..	
3	7-4-20	15	6	1	..	
4	3-2-21	17	17	1	..	
Total		56	38	..	..	69%

Sixty-nine per cent. of wheat plants were infected when inoculated. The fungus penetrated and at the point of infection the leaves withered and broke. There was spore formation on both sides of leaves.

TABLE XV.
Cross-inoculations on barley.

No.	Date	No. of inoculations	No. of successful inoculations	Control	Control infected	REMARKS
1	7-4-20	15	5	1	..	
2	3-2-21	12	5	1	..	
	Total	27	10	..	..	37%

Jowar Helminthosporium can infect barley leaves but the infection is not vigorous. The spots formed stop growing after sometime and spore formation is not plentiful.

TABLE XVI.
Cross-inoculations on oats.

No.	Date	No. of inoculations	No. of successful inoculations	Control	Control infected	REMARKS
1	6-2-20	12	8	1	..	
2	7-4-20	10	3	1	..	
3	3-2-21	12	3	1	..	
	Total	34	14	..	..	41%

As in the case of barley, on oats also infection is not vigorous and the fungus stops growing in the tissue after some time.

These experiments show that *jowar Helminthosporium* can infect—

<i>Jowar</i> 79%	<i>Maize</i> 48%	<i>Sugarcane</i> 40%
<i>Wheat</i> 69%	<i>Oats</i> 41%	<i>Barley</i> 37%
<i>Rice</i> 13%	<i>Bajra</i> 0%	

From the above figures it will appear that about 50 per cent or more infection takes place on wheat and maize, and in the case of sugarcane, oats

and barley about 40 per cent. *Jowar Helminthosporium* has very little effect on rice, and *bajra* is immune to its attack.

4. Summary.

1. *Helminthosporium* is found on almost all cereals and sugarcane in India. The present paper deals with species on *Zea Mays* (maize) and *Sorghum vulgare* (*jowar*).

2. *H. turcicum* Pass. on maize and *jowar* is morphologically identical. Both strains show some difference in culture, and it has been observed that in Bihar *Helminthosporium* is found only on maize and not on *jowar*, while in the Punjab it is recorded only on *jowar*. It is these two facts that lead the writer to believe that the fungus on *jowar* is a different strain from that on maize.

3. *H. turcicum* Pass. on maize is found in almost all countries where maize is grown and in Bihar it is one of the important diseases of this crop. The fungus is found both on leaves and on male inflorescence and its parasitic nature is proved by inoculation experiments on both hosts.

4. *H. turcicum* Pass. can be grown on a large number of media but the perfect stage of this fungus has not been observed on any of the media. The fungus prefers a reaction of 0 to +5 Fuller's scale.

5. The infection takes place either through a stoma or by piercing the cuticle and entering the cell below.

6. Cross-inoculation experiments show that both the strains of *Helminthosporium* on maize and on *jowar* can infect maize, *jowar*, wheat, barley, oats and sugarcane. The inoculations on *bajra* give negative results and those on rice were doubtful.

LIST OF ILLUSTRATIONS.

PLATE I.

- Fig. 1. An infected portion of a maize leaf showing spots formed by *Helminthosporium turcicum* Pass. (natural size).
,, 2. A portion of male inflorescence of maize infected with *Helminthosporium* (natural size).
,, 3. A leaf of jowar showing spots due to *Helminthosporium* (natural size).

PLATE II.

- Fig. 1. *H. turcicum* Pass. on maize. Germination of a spore drawn after 48 hours. $\times 136$.
Figs. 2 & 3. *H. turcicum* Pass. on maize. Germination of a spore drawn after 18 hours. $\times 440$.
Fig. 4. *H. turcicum* Pass. on maize. Germination of a spore drawn after 18 hours. $\times 640$.
,, 5. *H. turcicum* Pass. on maize. Germination of a spore drawn after 18 hours. A continuous tube is formed by the disappearance of septa and germ tube is coming out. $\times 640$.
,, 6. *H. turcicum* Pass. on maize. All the septa have been disorganized before the protrusion of germ tube. $\times 460$.
Figs. 7-13. *H. turcicum* Pass. Spores from Pusa maize leaf. $\times 460$.
,, 14-17. *H. turcicum* Pass. Spores from Burma maize leaves. $\times 460$.
,, 18 & 19. Conidiophores seen in a transverse section of an infected maize leaf. In Fig. 18, all the conidiophores are coming out by piercing an epidermal cell near a guard cell. In Fig. 19, a conidiophore coming out by piercing the epidermis and cuticle is shown. $\times 640$.
Fig. 20. Conidiophore. Section from a natural infected maize leaf. Conidiophore coming out by piercing the epidermis and cuticle. $\times 640$.
,, 21. A section showing conidiophores coming directly from an epidermal cell of a maize leaf. $\times 640$.
Figs. 22-25. Spores of *H. turcicum* Pass. from a diseased maize leaf from Almora $\times 700$.

PLATE III.

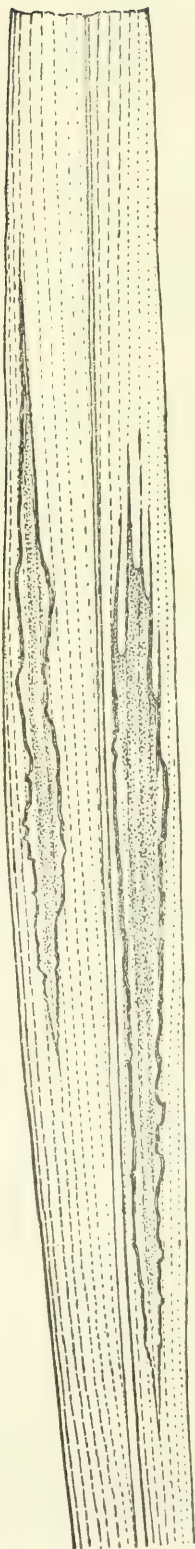
- Fig. 1. Maize *Helminthosporium*. Penetration of hypha through cell next to a guard cell of a stoma in maize. $\times 700$.
- „ 2. Maize *Helminthosporium*. Penetration through a stoma of maize. $\times 700$.
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- (Fig. 16. Tip of a conidiophore.)



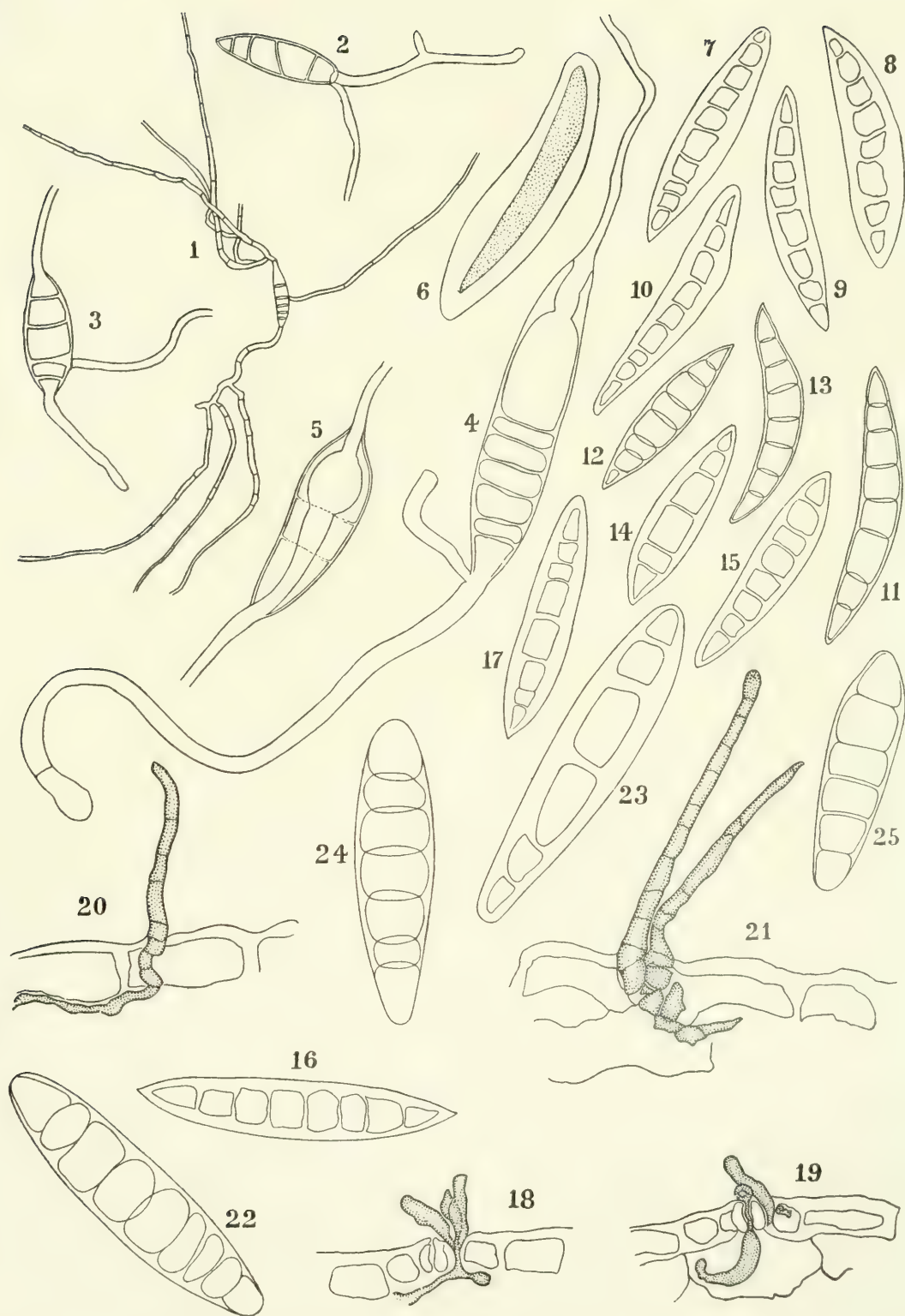
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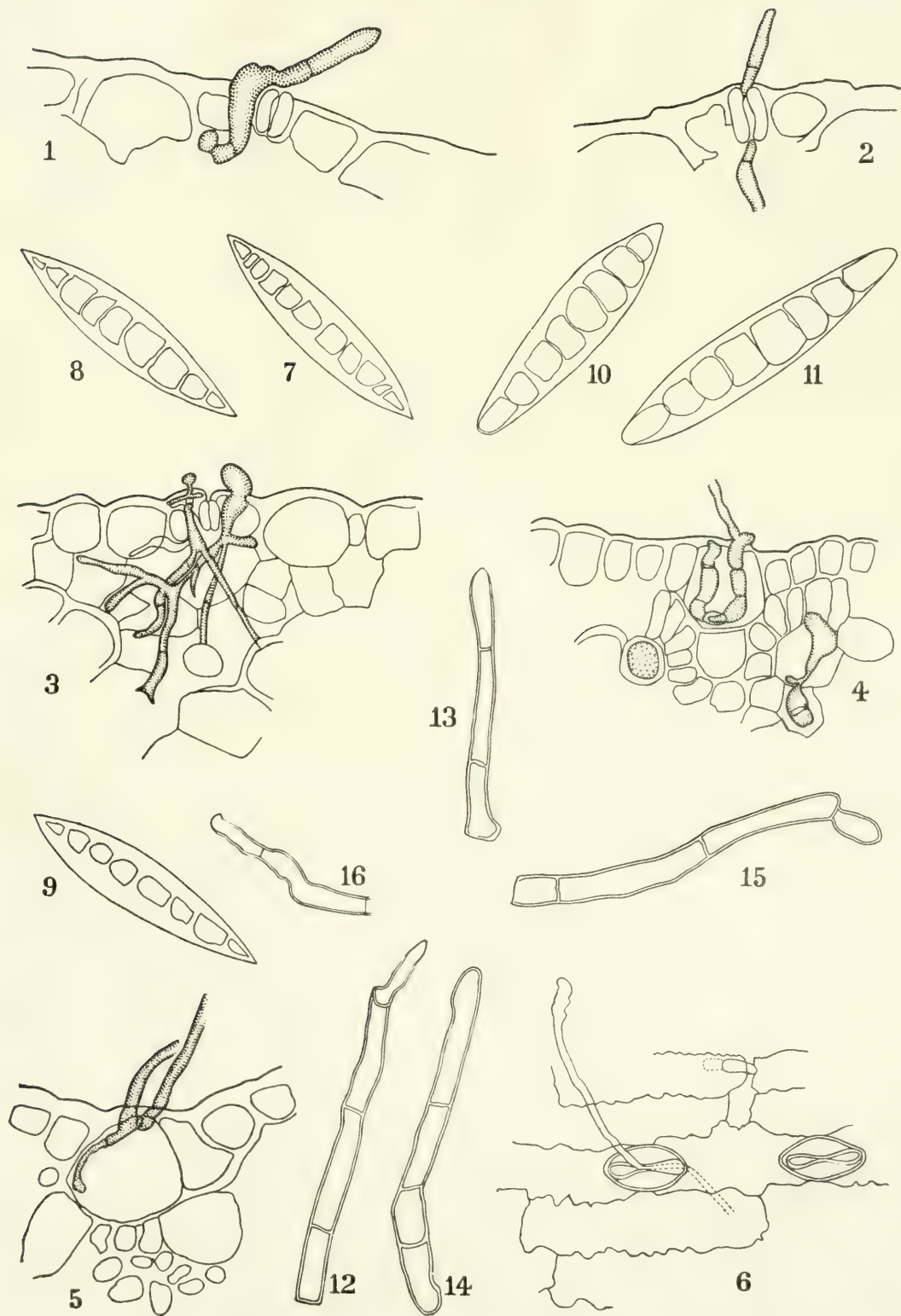
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